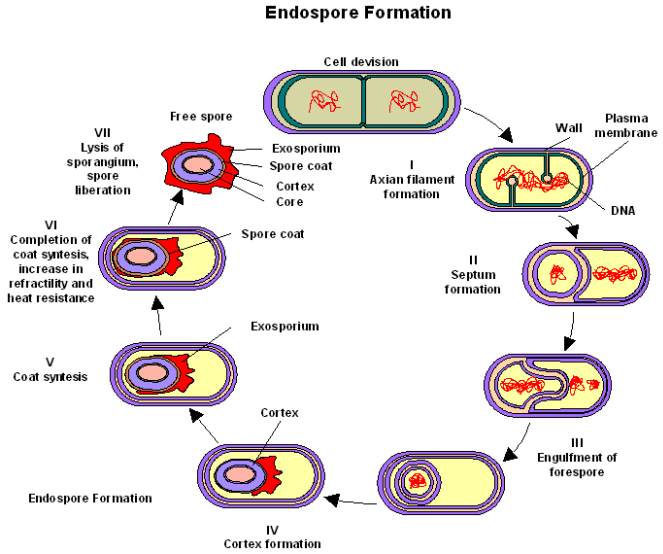
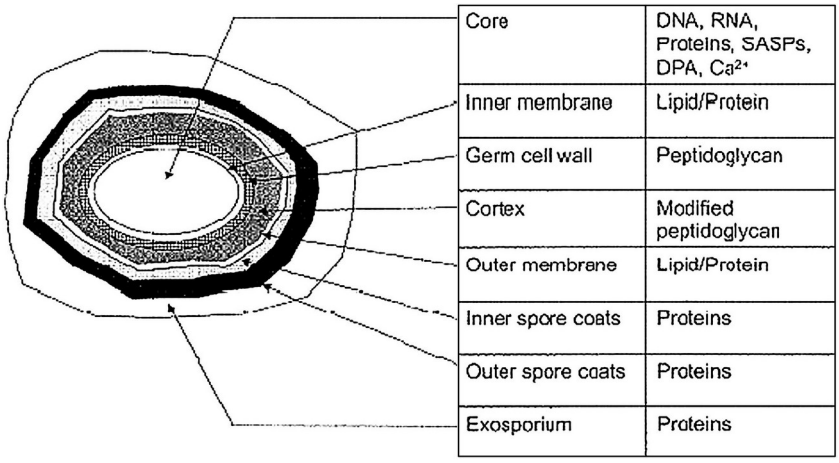
Endospore Staining Lab

Background and Introduction

The **endospore stain** is a differential stain that stains bacterial endospores. There are bacteria, which belong to the genera *Clostridium* and *Bacillus,* have the ability to produce metabolically inactive cells and are called spores. These spores are resistant to hostile environments that include extreme heat, no water, no nutrients and can even resist UV rays. A process called sporulation produces these spores. When their environment is stressed they will undergo sporulation. Please see the diagram below that illustrates the sporulation event timeline.



The endospore structure has multiple layers that enable it to be resistant to a variety of disinfectants, heat and radiation. The image below best describes what the endospore is composed of:



Core	DNA, RNA, Proteins, SASPs, DPA, Ca^{2+}
Inner membrane	Lipid/Protein
Germ cell wall	Peptidoglycan
Cortex	Modified peptidoglycan
Outer membrane	Lipid/Protein
Inner spore coats	Proteins
Outer spore coats	Proteins
Exosporium	Proteins

Pre-lab question:

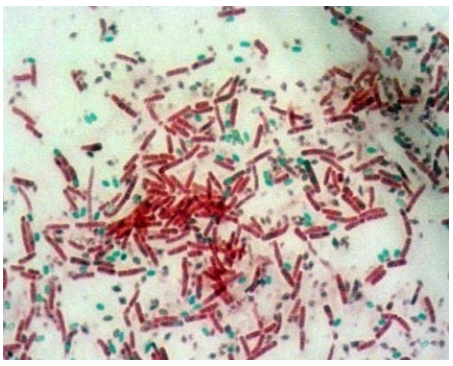
1. What would the endospore need in order for it to germinate and return to their vegetative state?

2. How does the endospore resist chemicals and UV radiation?

It is possible to see the endospores using a light microscope when they are stained correctly. The endospores can be in the middle of the cell(central), or at the end (terminal), or the end and the middle of the cells (subterminal). The endospores can have different shapes as well, like oval or round.

Staining information:

The **primary stain** in this procedure is **malachite green**. This has the ability to stain both the cell and the endospore. **Heat** is required to penetrate the thick endospore coat. Afterwards, the **decolorizer** is applied with water. This will remove the malachite green from all vegetative cells but not from endospores. The **counterstain** that is applied is **safranin** and will stain the decolorized vegetative cells pink, but not the endospore. Therefore, at the end of the endospore staining procedure, the endospores are green, and cells are pink/red. Below is an image that you should see underneath the microscope.



Post-lab question:

3. Why does the cell NOT look green after the completion of the entire procedure?

4. Why is heat used? What does the heat do to the cell?

Organisms: *Bacillus species*

Materials for Endospore Staining Lab

- 1 Wax Pencils

- 1 Distilled Water Bottle

- 1 Disinfectant Bottle

- 1 Test Tube Rack

- 1 Metal Inoculating Loop

- 1 Bunsen Burner and Hose


- Box of Microscope Slides (1 per bench)

- “Waste” 500ml Beakers (1 per bench)

- 1 Endospore Fast Staining Kits (2 per bench)
  - Each kit should have the following
    - Malchite Green
    - Safranin

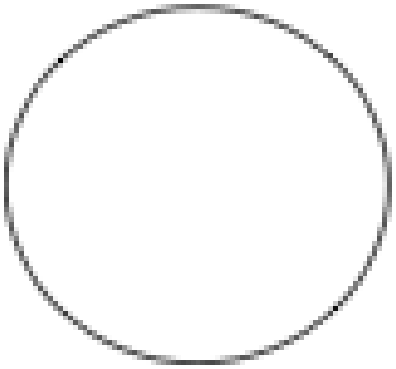
- Tupperware (3 per bench)

PROCEDURE:

Note: This is an individual lab exercise.

1. Remove all clutter and disinfect you bench
2. Make 2 THICK SMEARS of the *Bacillus species* on 2 clean slides.
3. Allow the slide to air dry and the heat fix.
4. While the smears are drying obtain a plastic dish and lid. In the bottom place two layers of paper towel and moisten them (be sure to remove excess moisture)
5. Place the heat fixed smears atop the moist paper towels. Cover the smears with two strips of paper towel, then flood the surface of these strips with malachite green
6. Snap the lid into place leaving one edge open for venting. Place the entire container in the microwave and heat for 1 minute at 70% power.
7. Remove the container from the microwave, and then at your bench top remove the slides from inside (please remember that HOT GLASS LOOKS LIKE COLD GLASS) and place it on the staining rack
8. Decolorize by running a stream of dH2O over the slide for at least 30 seconds. Remember to tilt the slide at a 45° angle.
9. Apply the safranin counter stain by flooding the surface of the smear and allowing this to sit for 1 minute.
10. Rinse the excess safranin away with dH_2O, and blot the slide dry with bibulous paper or Brown Paper Towels
11. Examine the spore stain under the microscope using oil immersion, paying close attention to the number of spores per field, cell shape, cellular arrangement, and relative size of the spores in relation to the vegetative cells.
12. Record all observations in your notebook along with representative drawings.

Data:



Bacillus species at 1000x

Post-Lab questions:

1. What would the results be if you used safranin as the primary stain and the malachite green as the counter stain?

2. What would the results be if you forgot to microwave?

3. Now that you know a little bit about spores why are diseases caused by spores a particular concern, and what makes them such attractive biological weapons?

4. Can you describe what an endospore is in your own words?